

# Association of PHB 1630 C>T and MTHFR 677 C>T polymorphisms with breast and ovarian cancer risk in BRCA1/2 mutation carriers: results from a multicenter study

**BACKGROUND:** The variable penetrance of breast cancer in *BRCA1/2* mutation carriers suggests that other genetic or environmental factors modify breast cancer risk. Two genes of special interest are prohibitin (*PHB*) and methylene-tetrahydrofolate reductase (*MTHFR*), both of which are important either directly or indirectly in maintaining genomic integrity.

**METHODS:** To evaluate the potential role of genetic variants within *PHB* and *MTHFR* in breast and ovarian cancer risk, 4102 *BRCA1* and 2093 *BRCA2* mutation carriers, and 6211 *BRCA1* and 2902 *BRCA2* carriers from the Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA) were genotyped for the *PHB* 1630 C>T (rs6917) polymorphism and the *MTHFR* 677 C>T (rs1801133) polymorphism, respectively.

**RESULTS:** There was no evidence of association between the *PHB* 1630 C>T and *MTHFR* 677 C>T polymorphisms with either disease for *BRCA1* or *BRCA2* mutation carriers when breast and ovarian cancer associations were evaluated separately. Analysis that evaluated associations for breast and ovarian cancer simultaneously showed some evidence that *BRCA1* mutation carriers who had the rare homozygote genotype (TT) of the *PHB* 1630 C>T polymorphism were at increased risk of both breast and ovarian cancer (HR 1.50, 95%CI 1.10–2.04 and HR 2.16, 95%CI 1.24–3.76, respectively). However, there was no evidence of association under a multiplicative model for the effect of each minor allele.

**CONCLUSION:** The *PHB* 1630TT genotype may modify breast and ovarian cancer risks in *BRCA1* mutation carriers. This association need to be evaluated in larger series of *BRCA1* mutation carriers.

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Breast and ovarian cancers are among the most common malignancies diagnosed in women. The major inherited susceptibilities to breast and/or ovarian cancer are germline mutations in either *BRCA1* or *BRCA2*. Even though both *BRCA1* and *BRCA2* confer a high risk of disease, it is not identical for all mutation carriers, which suggests there are other genetic and environmental factors that are capable of modifying disease penetrance. The identification of additional genetic factors that

could modify disease expression in *BRCA1* or *BRCA2* mutation carriers is an important facet to improving risk assessment. Two genes of special interest are prohibitin (*PHB*) and methylenetetrahydrofolate reductase (*MTHFR*), both of which are important either directly or indirectly in maintaining genomic integrity, including cell cycle control, DNA synthesis and methylation (Roskams *et al*, 1993; Bagley and Selhub, 1998; Friso *et al*, 2002).

The *PHB* gene is located on human chromosome 17q21, a region that undergoes frequent loss of heterozygosity in familial and sporadic breast and ovarian cancers (White *et al*, 1991; Black *et al*, 1993; Nagai *et al*, 1994). The gene product is a 30-kD intracellular antiproliferative protein, which interacts with the retinoblastoma tumour suppressor protein to regulate E2F-mediated transcription (White *et al*, 1991; Wang *et al*, 1999). The 3' untranslated region (3'UTR) of the *PHB* gene encodes a tumour suppressive *trans*-acting regulatory RNA molecule that arrests cell proliferation between the G1 and S phases of the cell cycle in normal epithelial cells and tumour breast cell lines (Jupe *et al*, 1996a; Manjeshwar *et al*, 2003). A single-nucleotide polymorphism (SNP), a C-to-T transition at position 1630 in the 3UTR (rs6917) creates a variant, which lacks antiproliferative activity (Jupe *et al*, 1996b) and significantly reduces cell motility (Manjeshwar *et al*, 2004). The presence of the T allele was shown to cause inactivation of the bioactive rRNA resulting in the loss of its proapoptotic function and a subsequent risk of malignant growth (Manjeshwar *et al*, 2003), and was reported to be associated with significantly increased risk of breast cancer in women aged less than 50 years who had a first-degree relative with breast cancer (Jupe *et al*, 2001).

The *MTHFR* gene produces a key enzyme in folate metabolism that catalyses the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the primary circulating form of folate. This reaction is essential for both purine nucleotide biosynthesis and remethylation of homocysteine to methionine used in DNA methylation (Kim, 1999; Choi and Mason, 2002). Two functional SNPs in the *MTHFR* gene, 677 C>T (rs1801133) and 1298 A>C (rs1801131), both associated with reduced enzyme activity *in vitro* have been described. The *MTHFR* 677TT (homozygote) genotype results in 30% enzyme activity *in vitro* compared with the CC wild-type, whereas the *MTHFR* 1298 CC genotype has been found to result in 60% enzyme activity *in vitro* compared with the AA wild-type (Frosst *et al*, 1995; Weisberg *et al*, 1998; Weisberg *et al*, 2001). Reduction of the *MTHFR* enzyme activity may result in cancer risk increase through impaired DNA repair synthesis and disruption of DNA methylation. In addition, it has been suggested that breast carcinogenesis could be associated with alteration of oestrogen receptor gene methylation patterns (Nass *et al*, 2000) and global DNA methylation (Soares *et al*, 1999). The association of *MTHFR* 677 C>T and 1298 A>C polymorphisms with breast cancer risk have been investigated and results of meta-analyses have shown a statistically significant association of the *MTHFR* 677 C>T polymorphism with breast cancer risk (Macis *et al*, 2007; Zhang *et al*, 2010; Qi *et al*, 2010).

Recently, the *PHB* 1630 C>T SNP was shown to be associated with a twofold increased breast cancer risk in Polish *BRCA1* mutation carriers of the CT, TT and combined CT + TT genotypes (Jakubowska *et al*, 2007a). Similarly the *MTHFR* 677C>T SNP was associated with a two to threefold increased risk of breast and ovarian cancer in the same population (Jakubowska *et al*, 2007b).

In the current study we have evaluated both associations in a large series of *BRCA1* and *BRCA2* mutation carriers from the Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA) (Chenevix-Trench *et al*, 2007).

## MATERIALS AND METHODS

### Patients

Eligible study subjects were women who carried a deleterious germ line mutation in *BRCA1* or *BRCA2* and were 18 years old or older. Information on study subjects was submitted by centres participating in CIMBA. Details of the CIMBA initiative, information about the participating centres and detailed inclusion criteria for

subjects can be found elsewhere (Chenevix-Trench *et al*, 2007). Briefly, collected data included year of birth, mutation description, family membership, ethnicity, country of residence, age at last follow-up, ages at breast and ovarian cancer diagnosis, and information on bilateral prophylactic mastectomy and prophylactic oophorectomy. Related individuals were identified through a unique family identifier. Only carriers of pathogenic mutations were included in the study. These were mutations generating a premature termination codon (frameshifts, small deletions and insertions, nonsense mutations, splice site mutations and large genomic rearrangements), large in-frame deletions that span one or more exons, deletions of transcription regulatory regions (promoter and/or first exons) expected to cause lack of expression of mutant allele and missense variants classified as pathogenic by Breast Cancer Information Core (BIC) or using the algorithms of Goldgar *et al* (2004) and Chenevix-Trench *et al* (2007). Truncating variants in exon 27 of *BRCA2* were excluded.

All analyses were restricted to mutation carriers of self-reported white European ancestry. A total of 4108 *BRCA1* mutation carriers, 2093 *BRCA2* mutation carriers derived from 13 centres participating in CIMBA were included in the analysis of rs6917 in *PHB* gene, and 7056 *BRCA1* mutation carriers and 3341 *BRCA2* mutation carriers from 23 centres in that of rs1801133 in *MTHFR* gene. The analysis included both related and unrelated mutation carriers in order to maximise the number of samples in the analysis.

All carriers participated in clinical or research studies at the host institutions under ethically approved protocols and data were analysed anonymously.

### Genotyping

Genotypes for the two polymorphisms rs6917 in *PHB* and rs1801133 in *MTHFR* were determined for each sample using PCR-RFLP (Jakubowska *et al*, 2007a,b), Taqman or iPLEX analyses (Table 1). The CIMBA genotyping quality control criteria, described in detail in <http://www.srl.cam.ac.uk/consortia/cimba/eligibility/eligibility.html>, were applied. Based on these criteria one study (169 carriers) was excluded due to low concordance rate for rs1801133, and 6 *BRCA1* carriers for rs6917 were excluded because of low number.

As an additional genotyping quality control assessment Hardy-Weinberg equilibrium (HWE) was evaluated in unrelated subjects for each polymorphism. There was no significant evidence of deviation from HWE except for one study (1115 carriers) for rs1801133 (HWE  $P$ -value =  $8 \times 10^{-6}$ ), so this was also excluded from the analysis. After all exclusions the rs6917 in *PHB* gene was analysed in 4102 *BRCA1* and 2093 *BRCA2* mutation carriers, and the rs1801133 in *MTHFR* gene in 6211 *BRCA1* and 2902 *BRCA2* mutation carriers (Table 1).

### Statistical analysis

The aim of the analysis was to evaluate the associations between the two polymorphisms and the risk of breast or ovarian cancer for *BRCA1* and *BRCA2* mutation carriers. For this purpose women were classified according to their age of cancer diagnosis or their age at last observation. Data were analysed within a retrospective likelihood framework by modelling the likelihood of the observed genotypes conditional on the disease phenotypes. This approach, described in detail elsewhere (Antoniou *et al*, 2007), adjusts for the fact that *BRCA1* and *BRCA2* mutation carriers were not randomly sampled with respect to their phenotype. Two types of analyses were carried out for each polymorphism. For the primary analysis, the associations with breast and ovarian cancer were evaluated separately for each disease. For the breast cancer risk association analysis, mutation carriers were censored at the age of the first breast cancer diagnosis, ovarian cancer diagnosis, bilateral

**Table 1** Number of BRCA1 and BRCA2 carriers by study group and genotyping platforms

| Study  | Country <sup>a</sup>    | BRCA1,<br>N | BRCA2,<br>N | Genotyping<br>Platform |
|--|-------------------------|-------------|-------------|------------------------|
| <b>PHB 1630 C&gt;T</b>   |                         |             |             |                        |
| Epidemiological study of BRCA1 & BRCA2 mutation carriers (EMBRACE)                               | UK and Eire             | 823         | 651         | iPLEX                  |
| Fox Chase Cancer Centre (FCCC)   | USA                     | 78          | 51          | iPLEX                  |
| Georgetown University  | USA                     | 32          | 15          |                        |
| Hereditary Breast and Ovarian study Netherlands (HEBON)  | The Netherlands         | 760         | 285         | iPLEX                  |
| Helsinki Breast Cancer Study (HEBCS)   | Finland                 | 103         | 104         | iPLEX                  |
| International Hereditary Cancer Centre (IHCC)  | Poland                  | 696         | 0           | PCR-RFLP               |
| Iceland Landspítali - University Hospital (ILUH)   | Iceland                 | 0           | 86          | iPLEX                  |
| Interdisciplinary Health Research International Team Breast Cancer Susceptibility (INHERIT BRCA) | Quebec -Canada          | 73          | 82          | Taqman                 |
| kConFab  | Australia               | 519         | 415         | iPLEX                  |
| Mayo Clinic (MAYO)   | USA                     | 214         | 115         | iPLEX                  |
| Pisa Breast Cancer Study (PBCS)  | Italy                   | 76          | 41          | iPLEX                  |
| Swedish Breast Cancer Study (SWE-BRCA)   | Sweden                  | 468         | 127         | iPLEX                  |
| University of Pennsylvania (UPENN)   | USA                     | 260         | 121         | iPLEX                  |
| Total  |                         | 4102        | 2093        |                        |
| <b>MTHFR677 C&gt;T</b>   |                         |             |             |                        |
| Spanish National Cancer Centre (CNIO)  | Spain, Greece           | 168         | 202         | Taqman                 |
| Deutsches Krebsforschungszentrum (DKFZ)  | Germany                 | 68          | 29          | Taqman                 |
| Epidemiological study of BRCA1 & BRCA2 mutation carriers (EMBRACE)                               | UK and Eire             | 642         | 469         | iPlex                  |
| Fox Chase Cancer Centre (FCCC)   | USA                     | 80          | 54          | iPlex                  |
| German Consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC)                              | Germany                 | 559         | 283         | Taqman                 |
| Genetic modifiers of cancer risk in BRCA1/2 mutation carriers (GEMO)                             | France, USA             | 1075        | 536         | iPlex                  |
| Hereditary Breast and Ovarian study Netherlands (HEBON)  | The Netherlands         | 480         | 0           | iPlex                  |
| Helsinki Breast Cancer Study (HEBCS)   | Finland                 | 101         | 103         | iPlex                  |
| International Hereditary Cancer Centre (IHCC)  | Poland                  | 686         | 0           | PCR-RFLP               |
| Interdisciplinary Health Research International Team Breast Cancer Susceptibility (INHERIT BRCA) | Quebec -Canada          | 72          | 82          | Taqman                 |
| kConFab  | Australia               | 409         | 322         | iPlex                  |
| Mayo Clinic (MAYO)   | USA                     | 106         | 56          | iPlex                  |
| Milan Breast Cancer Study Group (MBCSG)  | Italy                   | 246         | 132         | Taqman                 |
| Modifier Study of Quantitative Effects on Disease (ModSQuaD)                                     | Czech Republic, Belgium | 132         | 35          | Taqman                 |
| National Cancer Institute (NCI)  | USA                     | 139         | 57          | Taqman                 |
| National Israeli Cancer Control Center (NICCC)   | Israel                  | 221         | 129         | Taqman                 |
| Ontario Cancer Genetics Network (OCGN)   | Canada                  | 180         | 130         | Taqman                 |
| Odense University Hospital (OUH)   | Denmark                 | 109         | 0           | Taqman                 |
| Pisa Breast Cancer Study (PBCS)  | Italy                   | 56          | 32          | Taqman                 |
| Swedish Breast Cancer Study (SWE-BRCA)   | Sweden                  | 427         | 130         | Taqman                 |
| University of Pennsylvania (UPENN)   | USA                     | 255         | 121         | Taqman                 |
| Total  |                         | 6211        | 2902        |                        |

Abbreviations: MTHFR, methylene-tetrahydrofolate reductase; PHB, prohibitin; RFLP, restriction fragment length polymorphism. <sup>a</sup>Country of the clinic at which carriers are recruited

prophylactic mastectomy or the age at last observation. For this analysis, only mutation carriers censored at breast cancer were considered as affected. To evaluate the associations with ovarian cancer risk, carriers were censored at the age of ovarian cancer diagnosis, bilateral prophylactic oophorectomy or age at last observation, whichever occurred first. Only women censored at ovarian cancer diagnosis were considered as affected in this analysis. To allow for the fact that mutation carriers are at risk of developing both breast and ovarian cancer, in a second analysis we evaluated the associations between the SNPs with both breast and ovarian cancer simultaneously using a competing risk analysis, by estimating simultaneously HRs for both breast and ovarian cancers. Details of this method have been described elsewhere (Antoniou *et al*, 2010; Ramus *et al*, 2011; Barnes *et al*, 2012). A different censoring process was used in this case, whereby individuals were followed up to the age of the first breast or ovarian cancer diagnosis, and were considered to have developed the corresponding disease. No follow-up was considered after the first cancer diagnosis. Individuals were censored for breast cancer at the age of bilateral prophylactic mastectomy and for ovarian cancer at the age of bilateral oophorectomy, and were assumed to be unaffected for the corresponding disease. The remaining individuals were censored at the age at last observation and were assumed to be unaffected for both diseases.

All analyses were stratified by study group and country of residence, and used calendar-year and cohort-specific cancer incidences for BRCA1 and BRCA2 (Antoniou *et al*, 2008). A robust variance-estimation approach was used to allow for the non-independence among related carriers (Boos, 1992).

## RESULTS AND DISCUSSION

In this study, a total of 6195 individuals including 4102 BRCA1 and 2093 BRCA2 mutation carriers from 11 countries were eligible for inclusion in the analysis of the PHB 1630 C>T (rs6917) polymorphism (Table 1). The main analysis included all available mutation carriers, including the Polish BRCA1 mutation carriers used in the previous reports (Jakubowska *et al*, 2007a; Jakubowska *et al*, 2007b). There was no evidence of an association of rs6917 with breast or ovarian cancer risk for mutation carriers when the risks were evaluated separately (Table 2). However, the competing risk analysis, where associations were evaluated simultaneously for breast and ovarian cancer provided some evidence of association between the rare homozygote TT genotype with both breast cancer risk (HR 1.50, 95%CI 1.10–2.04) and ovarian cancer risk (HR 2.16, 95%CI 1.24–3.76) for BRCA1 mutation carriers (Table 3). The breast and ovarian cancer HRs for the TT genotype in the competing risk analysis were in the same direction as the



**Table 2** PHB 1630 C>T genotype frequencies by (a) disease status and breast cancer hazard ratio estimates; (b) disease status and ovarian cancer hazard ratio estimates

| Gene             | Genotype   | Unaffected<br>n (%) | Affected<br>n (%) | HR   | 95% CI    | P-value |
|------------------|------------|---------------------|-------------------|------|-----------|---------|
| (a) <sup>a</sup> |            |                     |                   |      |           |         |
| BRCA1            | CC         | 1443 (69.7)         | 1388 (68.3)       | 1.00 |           |         |
|                  | CT         | 575 (27.8)          | 574 (28.3)        | 1.04 | 0.92–1.18 |         |
|                  | TT         | 52 (2.5)            | 70 (3.4)          | 1.35 | 0.99–1.84 |         |
|                  | 2df test   |                     |                   |      |           | 0.17    |
|                  | Per-Allele |                     |                   | 1.08 | 0.97–1.21 | 0.15    |
| BRCA2            | CC         | 672 (67.9)          | 714 (64.7)        | 1.00 |           |         |
|                  | CT         | 293 (29.9)          | 354 (32.1)        | 1.15 | 0.96–1.37 |         |
|                  | TT         | 25 (2.5)            | 35 (3.2)          | 1.13 | 0.70–1.82 |         |
|                  | 2df test   |                     |                   |      |           | 0.29    |
|                  | Per-Allele |                     |                   | 1.12 | 0.96–1.30 | 0.14    |
| (b) <sup>b</sup> |            |                     |                   |      |           |         |
| BRCA1            | CC         | 2368 (68.9)         | 463 (69.6)        | 1.00 |           |         |
|                  | CT         | 972 (28.3)          | 177 (26.6)        | 0.93 | 0.83–1.06 |         |
|                  | TT         | 97 (2.8)            | 25 (3.8)          | 1.49 | 0.91–2.45 |         |
|                  | 2df test   |                     |                   |      |           | 0.18    |
|                  | Per-Allele |                     |                   | 1.03 | 0.87–1.23 | 0.73    |
| BRCA2            | CC         | 1274 (66.0)         | 112 (68.3)        | 1.00 |           |         |
|                  | CT         | 603 (31.3)          | 44 (26.8)         | 0.80 | 0.55–1.15 |         |
|                  | TT         | 52 (2.7)            | 8 (4.9)           | 1.63 | 0.67–3.99 |         |
|                  | 2df test   |                     |                   |      |           | 0.21    |
|                  | Per-Allele |                     |                   | 0.96 | 0.67–1.38 | 0.84    |

Abbreviation: PHB, prohibitin. <sup>a</sup>Diagnosed with breast cancer. <sup>b</sup>Diagnosed with ovarian cancer.

corresponding breast and ovarian cancer HR estimates in the analysis in which the breast and ovarian cancer associations were assessed separately (Table 2). The analyses that investigated the breast and ovarian cancer risk associations separately yielded no evidence of association with the TT genotype. When evaluating the associations with a single disease (breast or ovarian) in the primary analysis, individuals who developed the other disease were assumed to be unaffected in the analysis (i.e. treated as ‘controls’). Under this analysis, a potential bias could arise if PHB 1630 C>T is associated with both breast and ovarian cancer: if the magnitude of the true breast and ovarian cancer relative risks conferred by PHB 1630 C>T are in the same direction, then such an analysis could lead to an attenuation of the estimated associations (Barnes *et al*, 2012). Therefore, a plausible explanation for the apparent discrepancy between the two analyses could be due to this source of bias. However, the number of BRCA1 mutation carriers with the PHB 1630 TT genotype is limited and larger studies will be required to clarify this. The association with the TT genotype remained significant after excluding the Polish samples from the previously published study (Table 3).

A total of 6211 BRCA1 and 2902 BRCA2 participants from 16 countries were assessed for the associations between the common polymorphism 677C>T in MTHFR (rs1801133) and breast or ovarian cancer risk for women who harboured a germline mutation in either BRCA1 or BRCA2 (Table 1). When breast and ovarian cancer associations were evaluated separately (Table 4) or simultaneously (competing risk analysis) (Table 3), there was no evidence of association between the polymorphism with either disease for BRCA1 or BRCA2 mutation carriers. This observation is in contrast to previous findings in smaller studies of BRCA1 mutation carriers (Gershoni-Baruch *et al*, 2000; Pepe *et al*, 2007).

**Table 3:** PHB 1630 C>T and MTHFR 677 C>T genotype frequencies by disease status, BRCA1/2 mutation and, breast and ovarian cancer hazard ratio estimates in competing risk analysis

|                      | Unaffected n (%) | Ovarian cancer n (%) | HR          | 95% CI           | P-value | Breast cancer n (%) | HR          | 95% CI           | P-value |
|----------------------|------------------|----------------------|-------------|------------------|---------|---------------------|-------------|------------------|---------|
| PHB 1630 C>T         |                  |                      |             |                  |         |                     |             |                  |         |
| BRCA1                |                  |                      |             |                  |         |                     |             |                  |         |
| CC                   | 1155 (69.8)      | 334 (69.2)           | 1.00        |                  |         | 1342 (68.3)         | 1.00        |                  |         |
| CT                   | 465 (28.1)       | 130 (26.9)           | 0.99        | 0.78–1.25        |         | 554 (28.2)          | 1.04        | 0.92–1.19        |         |
| TT                   | 34 (2.1)         | 19 (3.9)             | <b>2.16</b> | <b>1.24–3.76</b> |         | 69 (3.5)            | <b>1.50</b> | <b>1.10–2.04</b> |         |
| Per-allele           |                  |                      | 1.16        | 0.93–1.41        | 0.19    |                     | 1.11        | 0.99–1.23        | 0.06    |
| BRCA1 excluding IHCC |                  |                      |             |                  |         |                     |             |                  |         |
| CC                   | 933 (67.9)       | 233 (65.8)           | 1.00        |                  |         | 1136 (67.7)         | 1.00        |                  |         |
| CT                   | 412 (30.0)       | 103 (29.1)           | 0.98        | 0.75–1.29        |         | 478 (28.5)          | 0.99        | 0.86–1.14        |         |
| TT                   | 29 (2.1)         | 18 (5.1)             | <b>2.32</b> | <b>1.34–4.05</b> |         | 64 (3.8)            | <b>1.43</b> | <b>1.05–1.98</b> |         |
| Per-allele           |                  |                      | 1.18        | 0.94–1.48        | 0.15    |                     | 1.07        | 0.95–1.20        | 0.27    |
| BRCA2                |                  |                      |             |                  |         |                     |             |                  |         |
| CC                   | 616 (67.1)       | 83 (71.6)            | 1.00        |                  |         | 687 (64.9)          | 1.00        |                  |         |
| CT                   | 282 (30.7)       | 28 (24.1)            | 0.69        | 0.45–1.07        |         | 337 (31.8)          | 1.08        | 0.90–1.30        |         |
| TT                   | 20 (2.2)         | 5 (4.3)              | 1.46        | 0.44–4.81        |         | 35 (3.3)            | 1.24        | 0.81–1.89        |         |
| Per-Allele           |                  |                      | 0.86        | 0.53–1.39        | 0.54    |                     | 1.09        | 0.94–1.27        | 0.23    |
| MTHFR 677 C>T        |                  |                      |             |                  |         |                     |             |                  |         |
| BRCA1                |                  |                      |             |                  |         |                     |             |                  |         |
| CC                   | 976 (43.3)       | 349 (43.1)           | 1.00        |                  |         | 1344 (42.7)         | 1.00        |                  |         |
| CT                   | 1000 (44.4)      | 361 (44.6)           | 0.95        | 0.79–1.13        |         | 1425 (45.3)         | 0.99        | 0.89–1.09        |         |
| TT                   | 279 (12.4)       | 99 (12.2)            | 0.93        | 0.72–1.21        |         | 378 (12.0)          | 0.96        | 0.82–1.13        |         |
| Per-allele           |                  |                      | 0.96        | 0.85–1.08        | 0.51    |                     | 0.98        | 0.99–1.06        | 0.63    |
| BRCA2                |                  |                      |             |                  |         |                     |             |                  |         |
| CC                   | 471 (43.0)       | 74 (42.1)            | 1.00        |                  |         | 688 (42.2)          | 1.00        |                  |         |
| CT                   | 481 (43.9)       | 85 (48.3)            | 1.09        | 0.76–1.57        |         | 741 (45.4)          | 1.05        | 0.90–1.23        |         |
| TT                   | 143 (13.1)       | 17 (9.7)             | 0.74        | 0.41–1.34        |         | 202 (12.4)          | 0.95        | 0.76–1.20        |         |
| Per-allele           |                  |                      | 0.93        | 0.72–1.20        | 0.57    |                     | 1.00        | 0.90–1.11        | 0.98    |

Abbreviations: MTHFR, methylene-tetrahydrofolate reductase; PHB, prohibitin. Significant results are marked in bold.

**Table 4** MTHFR 677 C>T genotype frequencies by (a) disease status and breast cancer hazard ratio estimates; (b) disease status and ovarian cancer hazard ratio estimates

| Gene             | Genotype   | Unaffected<br>n (%) | Affected<br>n (%) | HR   | 95% CI    | P-value |
|------------------|------------|---------------------|-------------------|------|-----------|---------|
| (a) <sup>a</sup> |            |                     |                   |      |           |         |
| BRCA1            | CC         | 1281 (43.3)         | 1388 (42.7)       | 1.00 |           |         |
|                  | CT         | 1313 (44.3)         | 1473 (45.3)       | 1.00 | 0.90–1.10 |         |
|                  | TT         | 367 (12.4)          | 389 (12.0)        | 0.96 | 0.82–1.12 |         |
|                  | 2 df test  |                     |                   |      |           | 0.84    |
|                  | Per-Allele |                     |                   | 0.98 | 0.92–1.05 | 0.64    |
| BRCA2            | CC         | 530 (43.3)          | 703 (41.9)        | 1.00 |           |         |
|                  | CT         | 542 (44.3)          | 765 (45.6)        | 1.05 | 0.91–1.22 |         |
|                  | TT         | 152 (12.4)          | 210 (12.5)        | 1.02 | 0.82–1.27 |         |
|                  | 2 df test  |                     |                   |      |           | 0.78    |
|                  | Per-Allele |                     |                   | 1.02 | 0.93–1.13 | 0.66    |
| (b) <sup>b</sup> |            |                     |                   |      |           |         |
| BRCA1            | CC         | 2190 (43.3)         | 479 (42.6)        | 1.00 |           |         |
|                  | CT         | 2259 (44.7)         | 527 (45.8)        | 1.01 | 0.88–1.16 |         |
|                  | TT         | 610 (12.1)          | 146 (12.7)        | 1.04 | 0.84–1.28 |         |
|                  | 2 df test  |                     |                   |      |           | 0.88    |
|                  | Per-Allele |                     |                   | 1.02 | 0.92–1.12 | 0.73    |
| BRCA2            | CC         | 1132 (42.7)         | 101 (40.7)        | 1.00 |           |         |
|                  | CT         | 1193 (44.9)         | 114 (46.0)        | 1.05 | 0.75–1.41 |         |
|                  | TT         | 329 (12.4)          | 33 (13.3)         | 1.14 | 0.74–1.76 |         |
|                  | 2 df test  |                     |                   |      |           | 0.84    |
|                  | Per-Allele |                     |                   | 1.06 | 0.87–1.30 | 0.57    |

Abbreviation: MTHFR, methylene-tetrahydrofolate reductase. <sup>a</sup>Diagnosed with breast cancer. <sup>b</sup>Diagnosed with ovarian cancer.

In this multicentre study we were unable to confirm the modifying effect of the MTHFR 677C>T polymorphism on breast cancer risk for BRCA1 carriers. We also did not detect an association of the above polymorphism with breast and/or ovarian cancer risk for BRCA2 mutation carriers. Previous studies were restricted to specific populations, and may potentially represent population specific effects (Gershoni-Baruch *et al*, 2000; Jakubowska *et al*, 2007b; Pepe *et al*, 2007; Beetstra *et al*, 2008). This explanation is confirmed by the fact that the frequency of 677TT genotype was substantially different between studies: 8.5% (52 in 609 carriers) in the Polish study (Jakubowska *et al*, 2007b), 13.5% (5 in 37 carriers) in a small study from Australia (Beetstra *et al*, 2008), 17% (82 in 484 carriers) in an Italian study (Pepe *et al*, 2007) and 21% (43 in 205 carriers) among Jewish carriers (Gershoni-Baruch *et al*, 2000). It is also noticeable that in Polish and Jewish carriers the modifying effect of MTHFR 677C>T polymorphism was observed for 677T homozygotes, whereas in the Italian study an increased risk of breast cancer was detected in carriers of the 677T allele. The genotype frequency of MTHFR 677CTT in this multi-population study was 12.2% with an equal distribution in breast cancer patients, ovarian cancer patients and unaffected individuals (12.1, 12.8 and 12.2, respectively). However, the most likely explanation could be the fact that all previous studies were based on much smaller data sets and associations based on those studies could represent false-positive findings. Although the present analysis has been performed in a much larger sample set of mutation carriers and therefore has a greater power to detect an association compared with any previously published studies it remains underpowered to identify weaker effects.

In conclusion, these findings show that in general the MTHFR 677C>T polymorphism is not likely to have an important role as modifier of breast and/or ovarian cancer risks in BRCA1/2 mutation carriers. There was some evidence that the PHB 1630 C>T polymorphism is associated with breast and ovarian cancer

risks in BRCA1 mutation carriers in the competing risk analysis, but this would need to be evaluated in additional analyses with larger number of mutation carriers. Future analyses should also aim to assess the associations with other clinical and tumour characteristics.

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## Conflict of interest

The authors declare no conflict of interest.

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